Journal of Cardiology 60 (2012) 180–186

Journal of Cardiology

journal homepage: www.elsevier.com/locate/jjcc

Original article

Links between sleep disordered breathing, coronary atherosclerotic burden, and cardiac biomarkers in patients with stable coronary artery disease

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ARTICLE INFO

Article history:
Received 18 October 2011
Received in revised form 29 February 2012
Accepted 13 March 2012
Available online 21 April 2012

Keywords:
Sleep disordered breathing
Coronary atherosclerosis
Troponin T
N-terminal pro-B-type natriuretic peptide
Cardiac biomarker

ABSTRACT

Background: Sleep disordered breathing (SDB) is highly prevalent in patients with cardiovascular disease, although it is not clear whether SDB has any link to coronary atherosclerotic burden in patients with stable coronary artery disease (CAD). This study sought to analyze the links between SDB, coronary atherosclerotic burden, and cardiac biomarkers in stable CAD patients.

Methods and results: We studied 83 consecutive patients who underwent coronary angiography or scheduled percutaneous coronary intervention. SDB was evaluated by an ambulatory polysomnographic monitoring device. Coronary atherosclerotic burden was evaluated by the Gensini score, and myocardial stress/injury were assessed by measuring plasma levels of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high sensitivity troponin T (hs-TnT). Patients with an apnea hypopnea index (AHI) $\geqq$ 15 events/h ($n = 32$) showed significantly higher Gensini score (35.7 ± 38.0 vs 20.1 ± 19.7, $p = 0.033$) than those with AHI < 15. The higher AHI group showed significantly higher NT-proBNP (275.8 ± 402.6 pg/ml vs 131.9 ± 146.3 pg/ml, $p = 0.047$) and hs-TnT levels (0.011 ± 0.005 ng/ml vs 0.008 ± 0.003 ng/ml, $p = 0.015$). Furthermore it was revealed that AHI significantly correlated with the Gensini score ($r = 0.253$, $p = 0.036$), NT-proBNP ($r = 0.266$, $p = 0.027$), and hs-TnT ($r = 0.274$, $p = 0.023$), and multiple stepwise linear regression analysis revealed that AHI ($\beta = 0.257$, $p = 0.029$) and history of smoking ($\beta = 0.244$, $p = 0.038$) were independently correlated with Gensini score among clinical and SDB-related parameters.

Conclusions: Severity of SDB has a significant link to the severity of coronary atherosclerotic burden, which also reflected elevated NT-proBNP and hs-TnT as silent myocardial ischemia and minute myocardial injury even in stable CAD patients.

Introduction

Sleep disordered breathing (SDB) has a higher prevalence in the population with cardiovascular disease, which seems to link to the pathophysiology of the disease. The prevalence of SDB, defined as an apnea-hypopnea index (AHI) of 5 or higher, has been estimated at 24% for men and 9% for women in the general population [1]. SDB has a major impact on patients with systemic hypertension, coronary artery disease (CAD), atrial fibrillation, etc. [2].

Obstructive sleep apnea (OSA), which is frequently observed among SDB, is characterized by periodic intermittent hypoxia followed by partial and complete pharyngeal collapse and cessation of breathing occurring during sleep. OSA leads to periodic intermittent hypoxia, exacerbation of negative intrathoracic pressure, and arousal, which leads to cardiovascular quiescence during non-rapid eye movement sleep to the storm condition by activating a cascade of hemodynamic, autonomic, inflammatory, and metabolic effects. The cycles of intermittent hypoxia and carbon-dioxide retention derange the balance between sympathetic and parasympathetic nervous regulation. This imbalance raises the heart rate with predominant sympathetic tone and systemic blood pressure (BP) with sympathetically mediated peripheral vasoconstriction [3]. Intermittent hypoxia activates inflammation, which impairs vascular endothelial function, and elevated inflammatory biomarkers, such as C-reactive protein [4] and oxidative stress

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0914-5087/$ – see front matter
http://dx.doi.org/10.1016/j.jjcc.2012.03.003

[5] play an important role in the pathogenesis and progression of atherosclerosis among CAD patients. OSA results in repetitive nocturnal hypoxia and sleep disturbance, which induces an increase in cytokine and inflammatory markers. Several studies [6–8] have shown that SDB is more prevalent in CAD patients and that it could provoke cardiovascular events. It has thus emerged as an independent risk factor in patients with CAD. However, there have been few reports investigating the impact of SDB on the severity of atherosclerotic plaque burden and cardiac biomarkers reflecting myocardial stress or injury in patients with stable CAD.

Methods

Study population

We conducted a cross-sectional, single-observational study. This study consisted of 83 consecutive (66 ± 11 years, male 87%) patients who were referred to the Nippon Medical School Chiba-Hokusoh Hospital for the evaluation and treatment of CAD. All patients underwent coronary angiography (CAG) or scheduled percutaneous coronary intervention (PCI) from September to December 2008. They attended sleep polygraphic monitoring for one night within one week before CAG or PCI. Major clinical exclusion criteria included significant renal dysfunction (creatinine $\geq$2 mg/dl), prior acute coronary syndrome or coronary artery bypass graft within the previous one month, symptomatic heart failure ($\geq$New York Heart Association class II) within the previous two weeks, severe valvular heart disease, cardiomyopathy, severe respiratory diseases with required oxygen inhalation. The study was approved by the Institutional Human Subjects Review Committee at Nippon Medical School Chiba-Hokusoh Hospital. Informed written consent was obtained from all participants.

Analysis of sleep apnea

The presence and severity of sleep apnea were determined by an overnight ambulatory cardiorespiratory monitoring device (Somté, Compumedics, Abbotsford, Victoria, Australia). Available signals include electrocardiograms, nasal airflow, thoracic and abdominal effort by breathing, percutaneous oxygen saturation (SpO_2), and pulse rate as previously reported [9–11]. Thoracoabdominal movements were recorded by respiratory inductance plethysmography. As the respiratory sensor, a thermistor was used to detect oronasal signals. SpO_2 was recorded by digital pulse oximetry (sampling frequency of 1 s). The oximeter signal quality of the cardiorespiratory monitoring device was proven to be valid in comparison with polysomnography [9,10]. All obtained signal data were manually analyzed with the following criteria to detect apnea and hypoxia. Apnea was defined as complete cessation of airflow for more than 10 s. Hypopnea was defined as a reduction of $\geq$50% in tidal volume from baseline for more than 10 s with oxygen desaturation of 4% from baseline [12]. The AHI (apnea-hypopnea index) was defined as the total number of apnea and hypopnea episodes per hour during sleep. The oxygen desaturation index (ODI) was the number of times per hour of sleep that the oxyhemoglobin saturation fell by $\geq$3%. OSA was diagnosed if complete cessation of oronasal flow occurred in the presence of thoracoabdominal breathing movements. We also analyzed the following data from the records, baseline SpO_2, lowest SpO_2, the longest apnea time, average heart rate during sleep, cumulative percentage time to total sleep time (%TST) at a pulse-oximetric oxygen saturation below 90% (%SpO_2 < 90%).

Coronary angiography and GENSINI score

CAG was performed according to standard techniques. Lumen narrowing <75% was considered as "non-significant CAD" and $\geq$75% as a significant stenosis. The coronary atherosclerotic burden was evaluated by coronary disease severity score based on a Gensini score method [13]. This score was developed with points assigned according to the category of severity of the stenosis (0 for 0–24%, 1 for 25–49%, 2 for 50–74%, 4 for 75–89%, 8 for 90–98%, 16 for 99%, and 32 for 100%) adjusting for lesion location, with more proximal lesions receiving a higher weighting factor. The Gensini score equals the sum of all segment scores. Each segment score equals the segment weighting factor multiplied by the severity score.

Measurement of hs-CRP, NT-proBNP and hs-TnT

Blood samples were drawn with an indwelling intra-artery catheter immediately before CAG or PCI. Blood samples were drawn in vacuum tubes containing lithium heparin, centrifuged, and the plasma was stored at $-70\,^{\circ}C$ for later measurements. The high-sensitivity C-reactive protein (hs-CRP) levels were measured with a latex turbidimetric immunoassay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels were determined with the use of a two-site electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics Ltd., Rotkreuz, Switzerland). High sensitivity troponin T (hs-TnT) measurements were performed in the research laboratory of Roche Diagnostics in Japan, using an electrochemiluminescence immunoassay (precommercial assay, Roche Diagnostics Ltd, Rotkreuz, Switzerland). This assay had an analytical range from 0.003 to 10 ng/ml, the 99th percentile value of 0.0135 ng/ml and the Coefficient Variance of 9% by using the Elecsys® (Roche) [14].

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package, version 14.0 (SPSS Inc., Chicago, IL, USA). Discrete variables are presented as counts and percentages. Continuous variables are presented as the means $\pm$ SD, as appropriate. Because hs-CRP levels have a skewed distribution, logarithmically transformed hs-CRP values were used in analyzing data. Comparisons between the two AHI groups were performed by the unpaired Student's *t*-test. The χ^2 or Fisher's exact test was used to compare nominal variables. To analyze the correlation between SDB parameters, the Gensini score, and biomarkers, Pearson correlation coefficients were calculated. Statistical significance was assumed at a *p*-value of <0.05. We defined the stepwise linear regression with explanatory variables which included age, sex, body mass index, systolic BP, left ventricular ejection fraction (LVEF), AHI, heart rate during sleep, history of smoking, estimated glomerular filtration rate (eGFR), low-density lipoprotein/high-density lipoprotein ratio, glycated hemoglobin A1c, log transformed hs-CRP as the model 1. In the model 2, Gensini score was added to the explanatory variables. The stepwise multiple linear regression analysis with model 1 was used to examine the independent association of the AHI levels with Gensini score. The stepwise multiple linear regression analyses with models 1 and 2 were used to examine and to reveal the independent interaction of AHI and Gensini score with the elevation of NT-proBNP and hs-TnT, respectively.

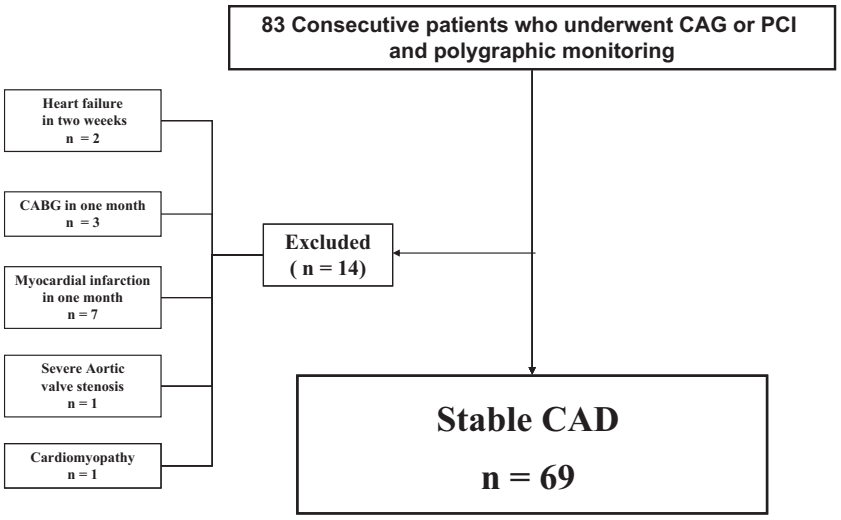

Fig. 1. Flow chart detailing number of all enrolled patients, excluded patients, and stable CAD patients. A total of 69 patients were finally analyzed after 14 patients were excluded from total 83 patients. CAG, coronary angiography; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; CAD, coronary artery disease.

Results

Patient backgrounds

A flow diagram detailing the number of patients undergoing the full set of CAG, polygraphic analysis, and analysis of cardiac biomarkers is presented in Fig. 1. We finally analyzed 69 patients with stable CAD after exclusion from 83 patients. The baseline characteristics of patients who completed polygraphic monitoring are shown in Table 1. The parameters of the sleep study in the studied patients showed that the mean AHI was 18.0 ± 11.9 events/h, the mean 3% ODI was 18.7 ± 12.4 events/h, and the mean minimum SpO_2 was 84.3 ± 7.7% (Table 2). The prevalence of AHI less than 5 was 9%, 5 ≤ AHI < 15 was 45%, 15 ≤ AHI < 30 was 27%, and

Table 1
Demographic and cardiovascular biomarkers in 69 coronary artery disease patients.

	Total	AHI < 15 (events/h)	AHI ≧ 15 (events/h)	*p*-value
n	69	37	32	
Age, years	66 ± 11	65 ± 11	67 ± 11	0.310
Male, *n* (%)	55 (80)	28 (76)	27 (84)	0.550
Abdominal circumference, cm	90 ± 9	88 ± 9	93 ± 7	0.019
Body mass index, kg/m^2	24.5 ± 3.5	23.7 ± 3.8	25.5 ± 3.0	0.033
Systolic blood pressure, mmHg	126 ± 16	125 ± 15	128 ± 17	0.495
Diastolic blood pressure, mmHg	68 ± 10	69 ± 10	68 ± 11	0.708
Heart rate, beats/min during sleep	60 ± 10	58 ± 9	62 ± 10	0.047
GENSINI Score	27.3 ± 30.4	20.1 ± 19.7	35.7 ± 38.0	0.033
LVEF, %	65 ± 11	67 ± 9	63 ± 13	0.105
Prior myocardial infarction, *n* (%)	27 (39)	13 (35)	14 (44)	0.621
Familial history, *n* (%)	23 (33)	12 (32)	11 (34)	0.533
History of hypertension, *n* (%)	47 (68)	25 (68)	22 (69)	0.562
History of diabetes, *n* (%)	27 (39)	11 (30)	16 (50)	0.070
History of hyperlipidemia, *n* (%)	60 (87)	33 (89)	27 (84)	0.406
History of smoking, *n* (%)	51 (74)	27 (73)	24 (75)	0.535
Lipid profile				
LDL-C, mg/dl	98 ± 25	93 ± 23	103 ± 26	0.108
HDL-C, mg/dl	48 ± 11	46 ± 9	50 ± 12	0.233
Non-HDL, mg/dl	125 ± 30	119 ± 28	132 ± 30	0.068
LDL/HDL ratio	2.1 ± 0.7	2.1 ± 0.7	2.2 ± 0.8	0.549
Renal profile				
eGFR, ml/min/1.73 m^2	70.5 ± 16.8	71.2 ± 13.9	69.7 ± 19.8	0.723
Urine albumin creatinine ratio	42.2 ± 133.7	19.9 ± 34.0	69.9 ± 194.9	0.136
Diabetes profile				
Fasting blood sugar, mg/dl	101 ± 19	99 ± 16	105 ± 22	0.168
HbA1c, %	5.8 ± 0.9	5.7 ± 0.8	5.9 ± 0.9	0.364
Biomarkers				
hs-CRP, ng/ml[a]	650.9 ± 3.6	537.0±3.9	813.5 ± 3.2	0.180
NT-proBNP, pg/ml	198.7 ± 300.7	131.9 ± 146.3	275.8 ± 402.6	0.047
hs-TnT, ng/ml	0.009 ± 0.004	0.008 ± 0.003	0.011 ± 0.005	0.015
NT-proBNP ≧ 1000 pg/ml, *n* (%)	4 (6)	0 (0)	4 (13)	0.042
hsTnT ≧ 0.014 ng/ml, *n* (%)	7 (10)	1 (3)	6 (19)	0.044

Values are mean ± SD or number of patients (%).
p-value shows the statistical significance between the below 15 and greater than equal to 15 of AHI.
LVEF, left ventricular ejection fraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; AHI, apnea-hypopnea index; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hsTnT, high sensitivity troponin T.
[a] Values are geometrized mean ± SD.

Table 2
Sleep disordered breathing-related parameters in 69 coronary artery disease patients.

	Total	AHI < 15 (events/h)	AHI ≧ 15 (events/h)	*p*-value
n	69	37	32	
AHI, events/h	18.0 ± 11.9	9.1 ± 3.5	28.3 ± 9.5	<0.001
Apnea index, events/h	7.6 ± 8.3	3.0 ± 2.8	12.9 ± 9.5	<0.001
Obstructive apnea index, events/h	6.9 ± 7.4	2.1 ± 2.4	10.3 ± 8.9	<0.001
Central apnea index, events/h	1.6 ± 2.2	0.8 ± 1.0	2.6 ± 2.8	0.001
Percent of obstructive apnea, %	72 ± 27	68 ± 28	75 ± 24	0.344
3% ODI, events/h	18.7 ± 12.4	10.8 ± 6.7	27.8 ± 11.2	<0.001
4% ODI, events/h	11.9 ± 10.0	5.8 ± 4.8	18.9 ± 9.8	<0.001
Longest obstructive apnea time, s	42 ± 28	33 ± 23	53 ± 28	0.001
Longest central apnea time, s	19 ± 11	15 ± 11	24 ± 11	0.001
Baseline SpO_2, %	96.0 ± 1.4	96.3 ± 1.2	95.6 ± 1.5	0.044
Minimum SpO_2, %	84.3 ± 7.7	88.0 ± 3.2	79.9 ± 9.0	<0.001
SpO_2 < 90%, % TST	2 ± 5	0.3 ± 0.5	4 ± 7	0.002

Values are mean ± SD.
p-value shows the statistical significance between the below 15 and greater than equal to 15 of AHI.
bpm, beats per minute; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; SpO_2, percutaneous oxygen saturation.
%TST, cumulative percentage time to total sleep time.

greater than or equal to 30 was 19% of the patients. Thus, in stable CAD patients, moderate to severe SDB prevalence was as high as approximately 50%.

Comparison of patients using an AHI of 15 as the cut-off

We divided the 69 patients into two groups, those with no or mild SDB (AHI < 15) and those with moderate to severe SDB (AHI ≥ 15). AHI ≥ 15 was defined as the cut-off value by the American Academy of Sleep Medicine [15]. Patients with AHI ≥ 15 ($n = 32$) showed significantly larger abdominal circumflex (93 ± 7 cm vs 88 ± 9 cm, $p = 0.019$) and higher body mass index (25.5 ± 3.0 kg/m^2 vs 23.7 ± 3.8 kg/m^2, $p = 0.033$) compared with those in the lower AHI group. The average heart rate during sleep was significantly higher in the higher AHI group (62 ± 10 bpm vs 58 ± 9 bpm, $p = 0.047$). In the coronary angiographic analysis, patients with AHI ≥ 15 showed significantly higher Gensini score compared with the lower AHI group (35.7 ± 38.0 vs 20.1 ± 19.7, $p = 0.033$). However the prevalence of hypertension, diabetes mellitus, and dyslipidemia were not significantly different in the two groups. Regarding the inflammatory and cardiac biomarkers, the levels of NT-proBNP and hs-TnT were significantly higher in the higher AHI group compared with the lower AHI group, however hs-CRP was not significantly different in the two groups. The proportion of patients with NT-proBNP levels ≧1000 pg/ml was significantly larger in the higher AHI group (0%.vs 13%, $p = 0.042$). Also the proportion of hs-TnT ≧0.014 ng/ml was significantly larger in the higher AHI group (3% vs 19%, $p = 0.044$) (Table 1). Thus, patients with moderate to severe SDB (AHI ≥ 15) suffered from more severe coronary atherosclerotic burden and also more myocardial stress and injury, independent of conventional risk factors such as age, gender, hypertension, diabetes mellitus, dyslipidemia, or smoking status.

Correlations between SDB parameters, severity of CAD and cardiac biomarkers

We investigated the relationship between SDB parameters and the severity of CAD assessed by the Gensini score and cardiac biomarkers. Across all patients, we found significant positive correlations between AHI and Gensini score ($r = 0.253$, $p = 0.036$), NT-proBNP ($r = 0.266$, $p = 0.027$), hs-TnT ($r = 0.274$, $p = 0.023$), and the average heart rate during sleep ($r = 0.291$, $p = 0.015$). The sum total time of apnea and hypopnea significantly correlated with Gensini score ($r = 0.244$, $p = 0.043$), NT-proBNP ($r = 0.283$, $p = 0.019$), hs-TnT ($r = 0.328$, $p = 0.006$), and the average heart rate during sleep ($r = 0.241$, $p = 0.046$), respectively (Fig. 2 and Table 3). Regarding

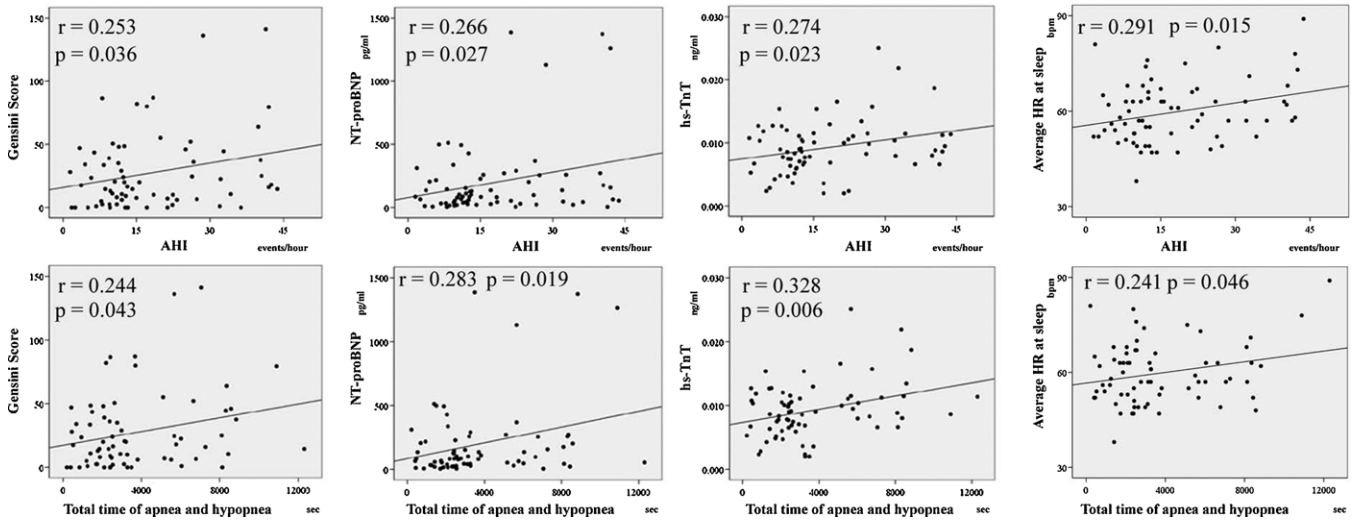

Fig. 2. Graphs showing correlations between sleep apnea parameters, Gensini score, and cardiac biomarkers. Correlations between sleep disordered breathing parameters and Gensini score, NT-proBNP, hs-TnT, and average heart rate during sleep. The upper graphs regard the apnea-hypopnea index and the lower graphs regard the sum total time of apnea and hypopnea. AHI, apnea-hypopnea index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hs-TnT, high sensitivity troponin T; HR, heart rate.

Table 3
Correlations between sleep disordered breathing-related parameters, Gensini score, and biomarkers.

	Gensini score		log hs-CRP		NT-proBNP		hs-TnT		HR during sleep	
	r	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value	*r*	*p*-value
Gensini score	–	–	0.102	0.404	0.266	0.027	0.349	0.003	0.036	0.771
AHI	0.253	0.036	0.191	0.115	0.266	0.027	0.274	0.023	0.291	0.015
The sum total time of apnea and hypopnea	0.244	0.043	0.174	0.152	0.283	0.019	0.328	0.006	0.241	0.046

hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide; hsTnT, high sensitivity troponin T; HR heart rate; AHI, apnea-hypopnea index.

Table 4
Multiple stepwise linear regression analysis to examine independent determinants of Gensini score among clinical and sleep disordered breathing-related parameters.

Gensini score		
	β	*p*
Smoking (yes = 1)	0.244	0.038
Apnea-hypopnea index	0.257	0.029
	(Model adjusted $R^2 = 0.097$)	

Age, sex, body mass index, systolic blood pressure, left ventricular ejection fraction, apnea-hypopnea index, heart rate during sleep, smoking, estimated glomerular filtration rate, low-density lipoprotein/high-density lipoprotein ratio, hemoglobin A1c, and log transformed high-sensitivity C-reactive protein were entered as the explanatory variables.
β: Standardized regression coefficient.

hs-CRP, a significant correlation was found with the average heart rate during sleep ($r = 0.280$, $p = 0.020$) among the various parameters of SDB.

A stepwise multiple linear regression analysis revealed AHI and smoking to be independent determinants of Gensini score (Table 4). Female gender, systolic BP, LVEF, and AHI were selected as the independent determinants of NT-proBNP, and eGFR and AHI were selected as those of hs-TnT in the model 1. When Gensini score was added to the explanatory variables, AHI turned out not to be independent and Gensini score was selected as the independent determinants of NT-proBNP and hs-TnT in model 2 (Table 5).

Discussion

The prevalence of SDB in patients with cardiovascular disease is known to be two- or three-fold greater than for populations without cardiovascular disease [16]. The Sleep Heart Health Study reported an independent association between SDB with AHI ≥ 11 and CAD and congestive heart failure [17]. The relative odds ratios were 1.27 and 2.38 respectively. Other studies have also revealed a significant link between severity of SDB and increased cardiovascular morbidity and mortality [6,18], however no studies have yet shown a detailed relationship between the severity of SDB and severity of coronary atherosclerotic burden based on CAG findings nor novel sensitive cardiac biomarkers in stable CAD patients.

Linkage between SDB and coronary atherosclerotic burden

In the present study, we divided the patients into two groups according to a cut-off AHI value of 15, namely non to mild SDB and moderate to severe SDB. We could not find any significant differences between the two groups with regard to conventional coronary risk factors; family history, hypertension, diabetes mellitus, lipid profile, or smoking. The lack of differences among the coronary risk factors between the two groups suggested that severity of SDB would contribute to the progression of coronary atherosclerosis independently, in addition to conventional coronary risk factors. The multiple linear regression analysis showed AHI to be independently associated with Gensini score and the positive correlations between the Gensini score and both AHI and sum total time of apnea and hypopnea. These findings proved that the severity of SDB significantly influenced the progression of the atherosclerotic burden. Intermittent hypoxia induced by SDB would accelerate the progression of atherosclerotic burden through many possible mechanisms, such as activation of sympathetic nerve activity, increasing inflammatory cytokines and oxidative stress [19]. Ryan et al. showed a preferential activation of inflammatory pathways mediated by the transcription factor nuclear factor

Table 5
Multiple stepwise linear regression analysis to examine independent determinants of NT-proBNP and hs-TnT among clinical and sleep disordered breathing-related parameters.

	Model 1[a]		Model 2[b]	
	β	*p*	*β*	*p*
NT-proBNP				
Female (yes = 1)	0.386	<0.001	0.388	<0.001
Systolic BP	0.343	0.001	0.362	<0.001
LVEF	−0.331	0.001	−0.316	0.002
AHI	0.211	0.036	–	–
Gensini score	Not included		0.230	0.022
	(Model adjusted $R^2 = 0.382$)		(Model adjusted $R^2 = 0.390$)	
High sensitive troponin T				
eGFR	−0.401	<0.001	−0.392	<0.001
AHI	0.266	0.016	–	–
Gensini score	Not included		0.332	0.002
	(Model adjusted $R^2 = 0.213$)		(Model adjusted $R^2 = 0.253$)	

β: Standardized regression coefficient.
NT-proBNP, N-terminal pro-B-type natriuretic peptide; BP, blood pressure; LVEF, left ventricular ejection fraction; AHI, apnea-hypopnea index; eGFR, estimated glomerular filtration rate; BMI, body mass index; LDL/HDL, low-density lipoprotein/high-density lipoprotein; Hb, hemoglobin; hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high sensitivity troponin T.
[a] Age, sex, BMI, systolic BP, LVEF, AHI, heart rate during sleep, smoking, eGFR, LDL/HDL ratio, HbA1c, and log transformed hs-CRP were entered as the explanatory variables.
[b] Variables used in the Model 1 plus Gensini score were entered as the explanatory variables.

kappa B (NF-κB) over adaptive hypoxia-inducible factor-1 (HIF-1) -dependent pathways in a cell culture model of intermittent hypoxia [20]. Intermittent hypoxia and reoxygenation in SDB with activation of NF-κB leads to the production of various pro-inflammatory mediators, such as tumor necrosis factor alfa, interleukin (IL)-6 or IL-8 which mediate the interaction of inflammation and endothelial dysfunction [21]. Hypoxemia mediated by intermittent apnea plays an important role in the inflammation cascade.

Secondary hypertension associated with SDB would be also involved in inducing the pathophysiological mechanisms. Elevated BP accompanied with SDB and intermittent hypoxia, activates chemoreceptors and the sympathetic nervous system [22] to induce endothelial dysfunction, arterial atherosclerosis, and artery remodeling. SDB predominantly increases BP during sleep, resulting in the non-dipping pattern or riser pattern of nocturnal BP. A characteristic finding of BP in subjects with SDB is increased nocturnal BP variability [23]. Periodic surges in BP, which occur at the same phase from later periods of apnea to the cessation of apnea, contribute to the shear stress on the vessels. These BP surges which occur more frequently would result in artery remodeling and elevated arterial stiffness, finally leading to progression of atherosclerosis. Patients with SDB had an elevated arterial stiffness, and Kato et al. reported that short-term continuous positive airway pressure (CPAP) therapy reduced atrial stiffness, as shown by the association of a reduction in cardio-ankle vascular index with the usage of CPAP in patients with moderate to severe SDB [24].

Linkage between SDB and cardiac biomarkers

To the best of our knowledge, we are the first to report that even in stable CAD patients, those with severe SDB showed silent myocardial stress detected by NT-proBNP and silent myocardial injury detected by the hs-TnT assay. A multiple stepwise linear regression analysis in model 2 revealed Gensini score to be independently associated with the NT-proBNP and hs-TnT levels. This indicated more severe atherosclerotic burden was independently associated with silent myocardial stress and minute myocardial injury in the stable CAD patients with SDB. The stable CAD patients with moderate and severe SDB showed significantly higher levels of NT-proBNP and larger percentage of NT-proBNP $\geqq$1000 pg/ml which had highly provable chronic heart failure in stage C and D with new classification of chronic heart failure updated in 2005 [25]. Emdin et al. reported that NT-proBNP concentrations increased from heart failure stage A to D by 107-fold [26]. The present study also revealed significant correlations between AHI and NT-proBNP. These findings would reflect the existence of silent myocardial ischemia or stress induced by more severe SDB. In the multiple stepwise linear regression analysis, female gender, systolic BP, and LVEF were found to be independent factors of NT-proBNP elevation. It was well known that these factors were associated with SDB [27,28]. In a community-based cohort, SDB was associated with reduced left ventricular systolic function [29]. These studies suggested that SDB would have indirect influence to the elevation of NT-proBNP and silent myocardial stress in stable CAD patients. The augmented cardiac afterload developed by activated sympathetic activity, the BP surge, and increased negative intrathoracic pressure would cause myocardial stress and the elevation of NT-proBNP.

It has also been found that patients with moderate and severe SDB showed significantly higher levels of hs-TnT compared with those with non and mild SDB. Furthermore they had a larger percentage of hs-TnT $\geqq$0.014 ng/ml which is the 99th percentile concentration of this hs-TnT assay [14] with cut-off value of newly developed classification for the universal definition of myocardial infarction [30]. In the multiple stepwise linear regression analysis, Gensini score and eGFR were found to be independent factors of hs-TnT. Our data suggested the presence of silent minute myocardial injury was evoked by more severe atherosclerotic burden associated with more severe SDB. Ndrepepa et al. reported that there was a close association between hs-TnT level and severity of CAD confirmed by angiographic atherosclerotic burden in patients with stable CAD [31]. The previous and our present observations suggest that the hs-TnT measurement enables the identification of subjects who have minute myocardial injury. Although Gami et al. reported that severe SDB patients with CAD did not show myocardial injury assessed by conventional cardiac troponin T assay (detection limit 0.01 ng/ml) [32], which is less sensitive compared with the present hs-TnT assay [33]. The present study applied a highly sensitive assay for cardiac troponin T permitting measurement of concentrations with an analytical range from 0.003 to 10 ng/ml which is to explain the ability to find an association between minute myocardial injury and atherosclerotic burden associated with severity of SDB in the present study. Apart from physical stress such as hemodynamic changes, periodic hypoxia followed by rapid reoxygenation might induce myocardial injury related to ischemia and reperfusion. The regional metabolic effects of hypoxia lead to intermittent increases in coronary blood flow during apnea, however in stable CAD patients the limited coronary flow reserve decreased the oxygen supply during apnea, resulting in myocardial ischemia. Hamilton et al. reported that the coronary blood flow reserve mismatched the demand of increased myocardial work [34] and this disturbed flow-metabolic coupling led to nocturnal myocardial ischemia and injury in stable CAD patients with SDB. We revealed that even in stable CAD patients, hs-TnT had a positive correlation with the AHI and sum total time of apnea and hypopnea. This supported the fact that the severity of SDB and nocturnal hypoxia are significantly related to the development of silent myocardial injury. Supporting this finding, Milleron et al. reported that treatment of OSA in CAD patients by CPAP was associated with a decrease in the occurrence of cardiovascular events [35]. Decreases in the AHI and nocturnal hypoxia would relieve silent myocardial stress and injury in patients with severe OSA, and decrease the occurrence of cardiovascular events.

Clinical implications and study limitations

The present findings suggest that the severity of SDB significantly links to the development of coronary atherosclerotic burden even in patients with stable CAD, and that silent myocardial stress and injury could be amplified by the presence of severe SDB, thus resulting in the elevation of NT-proBNP and hs-TnT.

In this study we did not measure nocturnal BP, pro-inflammatory cytokines, or adhesion molecules associated with inflammation to clarify the mechanism in detail. The sample size in the present study was not large, further study should be needed to confirm the correlation between coronary atherosclerotic burden, cardiac biomarkers, and SDB-related parameters. Furthermore investigation remains to be established to demonstrate therapeutic implication of SDB for the primary and secondary prevention of CAD.

Acknowledgement

Dr. Seino is a recipient of Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (20590843 and 29591077), and a part of the present investigation was supported by this grant.

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